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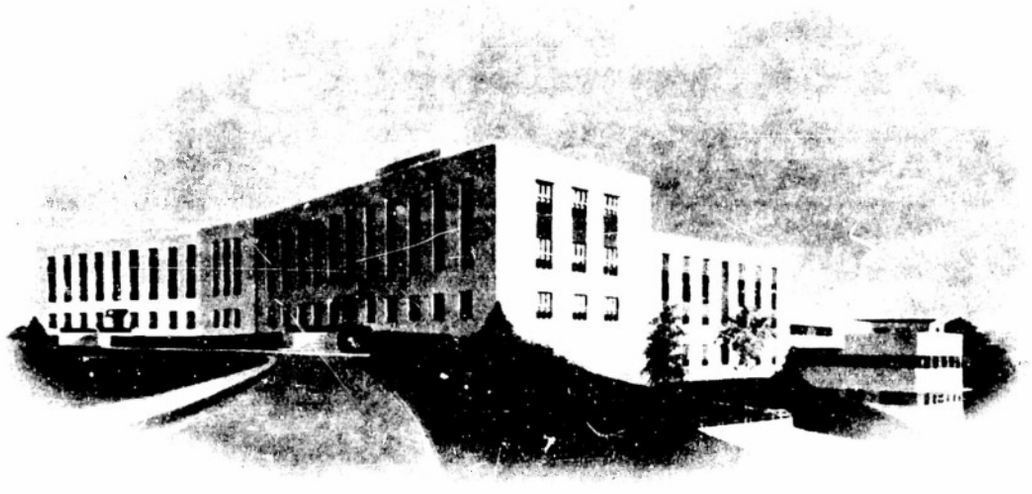

THE PERIPHERAL ORIGIN OF NERVOUS ACTIVITY IN THE VISUAL SYSTEM

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THE PERIPHERAL ORIGIN OF NERVOUS ACTIVITY IN THE VISUAL SYSTEM

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It is the function of the sense organs to reflect, in the nervous activity they generate, the state of the organism's environment, initiating chains of neural events that regulate behavior. The mechanisms whereby environmental influences excite activity in afferent nerve fibers have been discussed by many authors. Nevertheless, it is not yet possible to trace, step by step, the physical and chemical events that intervene between the action of a stimulus on a receptor and the response of the associated afferent fiber. This paper will consider some of the ideas that have been developed, and add new observations that bear on the problem of the origin of nervous activity, with particular reference to the visual system.[1]

The activity that is generated in afferent nerve fibers, when their sense organs are stimulated, consists of trains of nerve impulses such as are observed elsewhere in the nervous system. In any one fiber, the frequency of the discharge of impulses depends upon the intensity of the stimulus and upon the state of the receptor, as determined by the various factors affecting its responsiveness. These are now familiar facts of neurophysiology (Adrian, 1935). An example of such neural activity is given in Figure 1, which shows oscillograms of the action potentials recorded from a single optic nerve fiber from the eye of *Limulus*. In this case, a visual receptor element, stimulated by light, initiated the activity. As a result of the work of many investigators, beginning with Adrian and his associates, the discharge of impulses in afferent fibers has been recorded for almost all the major types of sense organs. The fact has emerged that, except for differences in the amount of sensory adaptation shown by different types of end organs under continuous stimulation, the patterns of response are essentially alike. Evidently, the various kinds of sensory end-organs with their associated afferent fibers possess certain fundamental properties in common.

[1] Work done under contract Nonr-248(11), between the Johns Hopkins University and the Office of Naval Research.

However, specificity of sensory pathway is essential to the discrimination of different kinds of stimuli. Each sense organ is especially sensitive to a particular stimulating agent. Although an important part of this specificity arises from the secondary structures of the various sense organs, much of it depends on specialized mechanisms in the receptors themselves. Thus one may expect to find two aspects to the study of the receptor mechanisms. On the one hand, general principles governing the excitation of all irritable tissue should be discernible, determining properties that are common to all sense organs and, indeed, to all nervous tissue. On the other hand, specific mechanisms concerned with the translation of particular external influences into sensory excitation may be expected to possess properties that differ widely from one type of receptor to another.

In the case of the visual system, the component that gives to the receptor its specific sensitivity to light is a photosensitive chemical compound contained in the structure of the visual end-organ. In the rods of the Vertebrate retina the specific photosensitive substance has been known for almost 100 years as visual purple, or rhodopsin. The biochemistry of extracted rhodopsin and other compounds related to it is being actively explored and much is understood about it. It is a conjugated protein in which the prosthetic group responsible for photosensitivity is a carotenoid closely related to vitamin A. When acted upon by light, rhodopsin undergoes a succession of chemical changes, ending in the liberation of the carotenoid fraction (retinene). Only the first step in this process is photochemical in nature, the rest are thermal. In the eye, visual purple is constantly being replenished by other chemical mechanisms. In part at least, these can be duplicated in the test tube, and considerable knowledge has been gained concerning the enzyme mechanisms involved. Not only has the photosensitive substance of the Vertebrate retinal rods been investigated, but that of the cones as well, and biochemical studies of visual pigments have been extended to a few Invertebrates. Some of the

recent developments in this field have been reviewed by Wald (1949, 1951), in whose laboratory many of the important advances have taken place.

The initial step in the action of light on the visual receptor is the absorption of energy from the incident radiation by the photosensitive substance. Not all of the radiation in the electromagnetic spectrum is "visible," and in the visible spectrum not all wave lengths are equally effective. This simply reflects the fact that the photosensitive substance of the visual receptor has an absorption spectrum that is not uniform. The more efficiently light of a given wave-length is absorbed by the visual receptors, the greater is the sensitivity at that wave-length. The absorption spectrum of rhodopsin has been measured, and, after suitable correction, has been found to explain satisfactorily the spectral distribution of sensitivity of the Vertebrate rods (see Wald, 1949).

Several other familiar properties of the visual system have been related directly to the chemistry of the photosensitive substance. In the excitation of the receptor, the familiar reciprocal relation between the intensity of the stimulating light and duration of the exposure has been attributed to this photochemical system (Hecht, 1919a; Hartline, 1934). This relation is looked upon simply as the expression of the Bunsen-Roscoe law of photochemistry. Furthermore, the very large changes in sensitivity during light and dark adaptation have been attributed to a decrease in the concentration of the photosensitive substance by photolysis, and to its regeneration by chemical mechanisms that are independent of light (Hecht, 1919b; Wald and Clark, 1937; Hartline and McDonald, 1947). Here it may be well to exercise caution in interpretation, for sensory adaptation is a universal property of receptors of all kinds. It scarcely seems reasonable to ignore this and ascribe all of the sensitivity changes in the visual receptor to alterations in its highly specialized photochemical component.

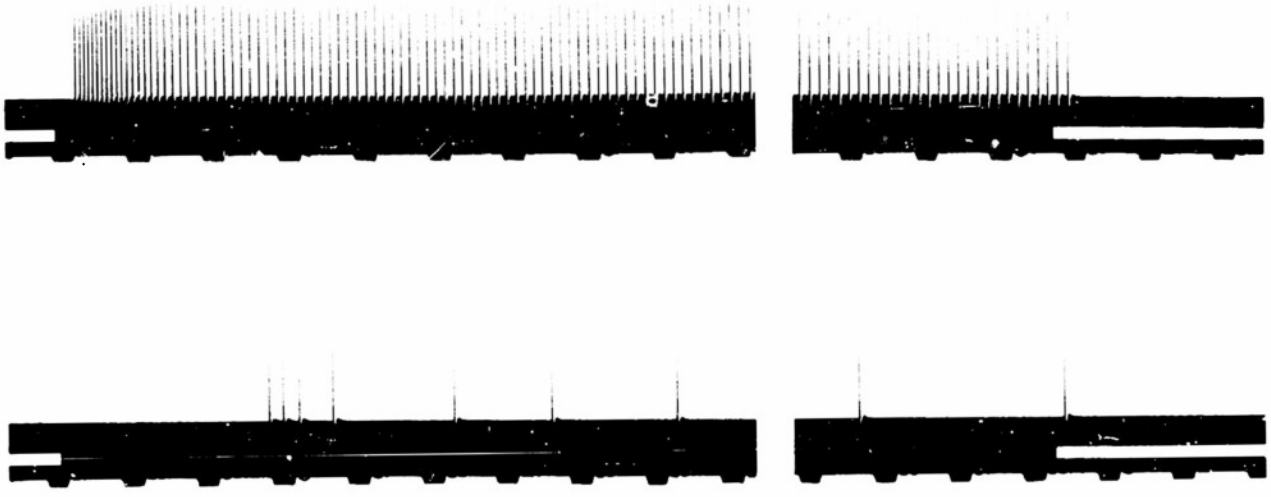

FIG. 1. Oscillograms of action potentials of a single optic nerve fiber of *Limulus*, in response to prolonged illumination of the eye. For the top record, the intensity of stimulating light was 10,000 times that used for the bottom record. Eye partially light adapted. Signal of exposure to light blackens out the white line above time marks. Each record interrupted for approximately 7 sec. Time marked in ½ sec.

The processes that intervene between the initial photochemical reaction in the receptor and the initiation of nervous activity are almost completely unknown. However, we have made some experimental observations relating to the time required for these processes. One of them concerns the persistence of excitatory effects in a photoreceptor after a brief exposure to light. As recorded in a single optic nerve fiber of *Limulus*, the entire discharge of impulses in response to a very short flash of light acting upon the receptor takes place after the flash is over, and often lasts for several tenths of a second (Fig. 2). A very intense flash can elicit a discharge lasting for a minute or more. Even after a flash of light too weak to elicit a response, excitatory effects can be shown to persist for one or two seconds (Wagman, Hartline and Milne, 1949). In the experiment illustrated in Figure 3 this was demonstrated by using a second flash to test the sensitivity of the receptor at various

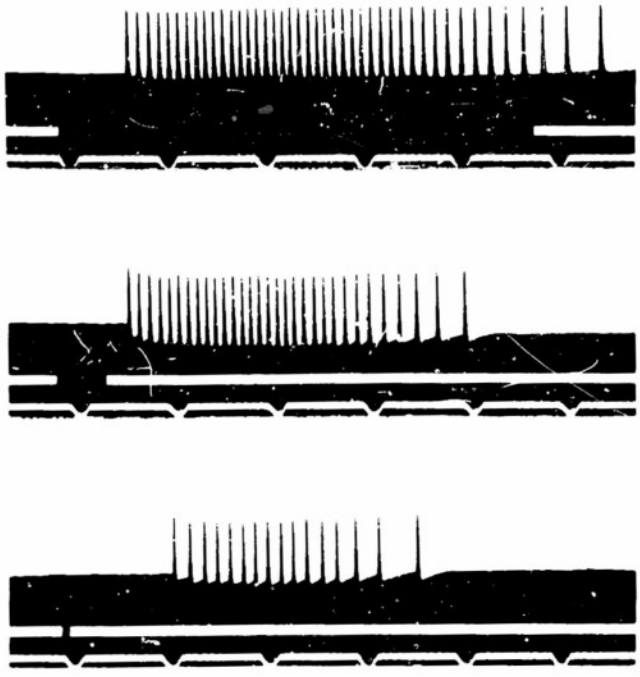

FIG. 2. Discharge of impulses in a single optic nerve fiber of *Limulus*, in response to exposures of the eye to light of the same intensity for durations of 0.97 sec, 0.096 sec and 0.010 sec (top to bottom). Signal marking period of exposure blackens out white line above time marks. Time in ¼ sec. The times from the onset of illumination to the successive occurrences of corresponding impulses in the upper and middle records were the same for the first 10 impulses. From the 11th impulse on, the occurrences were significantly earlier in the upper record than in the middle. Therefore, 0.096 sec was the "critical duration" for the 10th impulse, for which the time of occurrence = 0.32 sec. For the 1st impulse (time of occurrence = 0.14 sec) the critical duration at this intensity was 0.04 sec.

times after the exposure to the subliminal flash. (Following the period of enhanced excitability in this experiment there was a transitory period of diminished sensitivity. Except for the much longer time constants, these changes in excitability are reminiscent of those well known in peripheral nerve subjected to subliminal electrical shocks.) The persistence of the exciting effects of light can be ascribed either to the properties of the photochemical system of the receptor or, equally well, to later events in the process leading to the discharge of nerve impulses. However, these observations do show that the excitatory effects in the photoreceptor are not limited to the period during which light energy is being absorbed and active photolysis is taking place.

It has long been known that there is an interval of time between the absorption of light and the first sign of response by the organism. Many years ago Hecht (1919b) attributed most of this delay to a latent period in the photoreceptor itself, subsequent to photolysis. The analysis of the electrical response of isolated photoreceptors has shown his interpretation to be correct. When the discharge of impulses is recorded in nerve fibers coming directly from the receptor elements, as in the eye of *Limulus*, there is an interval of time between the onset of illumination and the beginning of the discharge (Fig. 1). At 18° C the latent period may range in value from several hundredths of a second at high intensities to one or two seconds, or even more, when the receptor is dark adapted and the intensity near threshold. Lowering the temperature slows the latent period very markedly. The response to a short flash (Fig. 2) also shows a latent period, so that, as

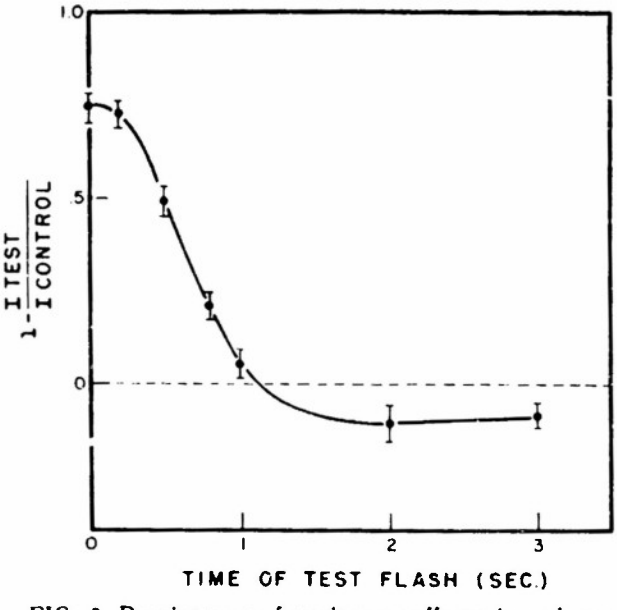

FIG. 3. Persistence of excitatory effects in a photoreceptor element in the eye of *Limulus* at various times following a short flash (0.02 sec duration) of light of subliminal intensity (70% of normal threshold). At various times after the subliminal flash (abscissae), the receptor was illuminated by test flashes (0.02 sec duration) the intensity of which was adjusted until the receptor would respond to 50% of the flashes. The amount by which the test flash had to be diminished from its normal threshold value was taken as the measure of the excitation remainder (ordinates). Normal threshold = 1 unit of intensity. After approximately 1 sec the remainder became negative (post-excitatory depression), after which the receptor recovered slowly, until at 15 sec its threshold had returned to normal. Each point is the weighted mean of several determinations; the limits of ± 1 standard error are indicated by the lengths of the vertical lines through the points. (From Hartline, Wagman, Wagner, and Milne, in preparation.)

we mentioned above, the entire response may take place when the receptor is in darkness. Evidently the products of photolysis that are generated during illumination take some time to exert their effects.

The latent period of the *Limulus* photoreceptor may be analyzed further. In the response to a short flash of fixed intensity the latent period decreases with increasing duration of the flash up to a certain critical value. Beyond this "critical duration" continuation of the exposure has no further effect on the time of appearance of the first impulse. It is as though the processes determining the beginning of the response are completed at the end of the critical duration, even though the impulses themselves do not appear until sometime later. This is very similar to the "sensitization period" described by Hecht for the response of *Mya*, although his theory fails to describe the *Limulus* data quantitatively.

This experimental analysis may be extended to the timing of the impulses that occur after the first one. Continuation of the exposure beyond the duration that is "critical" for the first affects later impulses, but for these, too, "critical durations" are observed that are longer in proportion to the times of appearance of the respective impulses (see Fig. 2). (This analysis has not been extended to the timing of impulses that occur later than those determining the maximum frequency of the discharge, usually 10–20 impulses). The significance of the "critical duration" for the present problem has been discussed at an earlier symposium (Hartline, 1935). Since then, we have found (in collaboration with Dr. J. H. Stover) that, for any one preparation, the "critical duration" for a given impulse is a nearly constant fraction (usually between ⅓ to ½) of the time at which that impulse appears in response to prolonged illumination, irrespective of conditions of temperature, adaptation and intensity of stimulation. The interval between the end of the "critical duration" for a given impulse, and the time of its appearance in the nerve discharge, being a constant fraction of that time, is shorter the higher the intensity of illumination. Whatever the process may be that occupies this final interval, it proceeds more rapidly the stronger the stimulus to the receptor, although it is independent of whether or not the light is shining on the receptor while it is taking place.

Another demonstration of the time lag between the stimulating light and the response it elicits is seen when the receptor, illuminated steadily and discharging a steady train of impulses, is subjected to a sudden increase (or sudden decrease) in light intensity. In response there is an increase (or decrease) in the frequency of the discharge (MacNichol and Hartline, 1948). This change begins after an appreciable latency (0.1 to 0.2 sec), during which time the original discharge rate is entirely unaltered.

These observations show that excitatory effects in the photoreceptor take time to develop to the point where they result in the discharge of nerve impulses. They suggest the concept of a photochemical stimulus distinct from subsequent reactions that finally excite the axon. These intervening processes limit the speed with which a photoreceptor can respond to a change in the stimulus. However, except to show that the processes involved consume time, such studies have contributed little to the understanding of the physical nature of the mechanisms whereby the products of photochemical action generate nerve activity. A more direct experimental approach is needed.

Nearly 100 years ago Holmgren discovered the retinal action potential. Since then, many investigators have studied the electrical responses to illumination that can be obtained from the eyes of a variety of animals merely by placing electrodes on either side of the layer of sensory elements and recording fluctuations in electric potential by a suitable instrument. Of the many treatises on this extensive subject, those of Kohlrausch (1931) and Granit (1947) may be recommended. An eye need not be especially highly developed to yield a simple retinal action potential (Fig. 4). It is only necessary that it be sufficiently well organized so that the sensory elements are similarly oriented and closely packed, presenting favorable electrical conditions for recording. It is now believed that a large component of the retinal action potential in all eyes arises from the sensory elements themselves, and that its behavior parallels closely the activity of these cells. For these reasons, and because of the importance of electrical phenomena in the initiation and propagation of nervous activity, it seems reasonable to hope that a study of the retinal action potential may provide a direct method of investigating the origin of nervous activity in the visual receptor.

This idea is made particularly inviting by a consideration of the polarity of the electrical response from a layer of visual receptor elements. In all eyes so far studied, a large component of the electrical response to light is usually in a direction indicating increased negativity of the

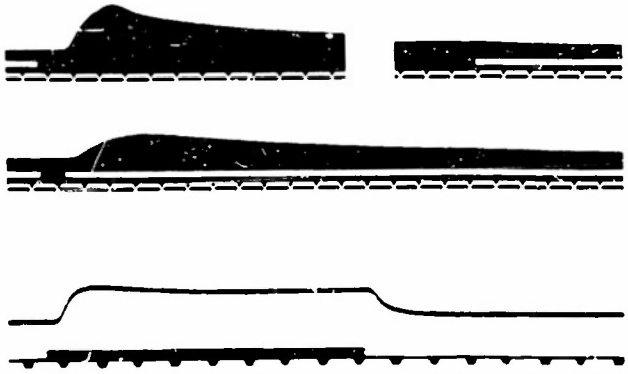

FIG. 4. Upper and middle records: Retinal action potentials recorded from an eye-spot of a star-fish (*Asterias* sp.). (Direct-coupled amplifier was used for this and for all other retinal action potentials recorded in this paper.) Leads: corneal surface to back of eye. Deflection upward indicates corneal lead becoming more negative with respect to lead on back of eye. Duration of exposure indicated by signal, which blackens the white line just above the time marks (interruption of upper record was for approx. 3 sec). Time in ⅕ sec. Lower record: Retinal action potential from an eye of a spider (species undetermined). Leads: surface of cornea to optic nerve. Deflection upward indicates increasing negativity of corneal lead relative to lead on nerve. Amplitude of initial maximum = 0.9 mv. Period of illumination signalled by heavy black band above time marks. Time in ⅕ sec.

free distal ends of the receptor cells with respect to the ends from which their nerve fibers arise. In peripheral nerve, the propagated impulse is associated with local negativity of the excited region. The direction of the local currents that are set up are then such as to cause spread of the excitation. It is tempting to postulate that as an ultimate consequence of the initial photochemical reaction a potential gradient is set up in the photoreceptor, causing local currents in the direction favorable to the spread of excitation in the receptor's nerve fiber. By this hypothesis a large component of the retinal action potential would be the external sign of an electrical event in the receptors that is one of the essential links in the chain of processes relating the action of light with a discharge of nerve impulses. This would be in line with ideas that are currently held relating the discharge of impulses to slow action potentials in other sense organs and in nerve cells.

Many authors have expressed or implied this interpretation of the significance of the retinal action potential, but there are difficulties to be met before this hypothesis can be accepted. In many highly developed eyes, as in the insects and Vertebrates, the receptor elements have associated with them extensive ganglionic structures, the activity of which may contribute notably to the retinal action potential (Fig. 5; see Granit, *loc. cit.*). Moreover, the afferent nerve activity to which the retinal potential is related, according to this hypothesis, should be recorded from fibers arising from the receptors rather than from more proximal neurones in which the activity may be affected by very complex interactions within the optic ganglia, as in the vertebrate retina. By using a simpler eye, or one in which the ganglionic structures can be removed or inactivated (Bernhard, 1942), some of these complications can be avoided. The visual receptors in an eye are often of diverse types, as for example the rods and cones in the vertebrates, or they may have a statistical distribution of their properties that would cloud an attempt to relate their nerve fiber activity to the retinal action potential. Ideally, it would be

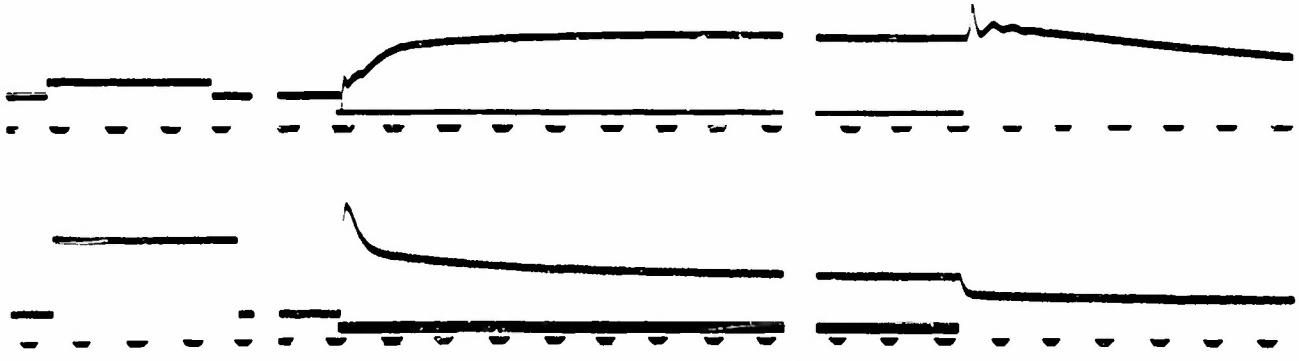

FIG. 5. Retinal action potential of the compound eye of the house fly. Upper record taken from the intact insect, leads: cornea to indifferent lead on head. Lower record taken after removal of the optic ganglion, leads: cornea to inner surface of sensory layer. Calibration: upper record 2.0 mv; lower record 0.5 mv. Deflection upward indicates increasing negativity of corneal lead. Time in ⅕ sec. Illumination signal indicated by black line just above the time marks.

desirable to record simultaneously, from a single receptor, both its action potentials and the activity in its nerve fiber. As yet this has not been possible. However, in the compound eye of *Limulus* we have been able to isolate intact single ommatidia, and in experiments on such preparations to record both the discharge of impulses in the nerve leading from the ommatidium and the slow action potential of the group of cells comprising its sensory structure (Hartline, 1948).

The structure of the eye of *Limulus* is not yet satisfactorily understood. Current studies by Waterman (1951) and some in our own laboratory (Miller, 1952) are making progress, and may help to clarify the uncertainties. Because the *Limulus* eye has been so favorable for physiological study, we will describe briefly what we know of its histology.

The compound, lateral eye of *Limulus* contains, on the average, some 600 ommatidia. The lenticular portion of each ommatidium is a thickening of the transparent chitinous cornea, forming a small conical projection into the interior of the eye. At the end of each cone, embedded in heavily pigmented tissue, is a cell-complex, the sensory portion of the ommatidium (see Fig. 6). There are several types of cells in this complex, of which two seem to be neuroepitheleal and concerned with the light receptive process. One of these, generally termed the retinula cell, is a fairly large cell, some 150 μ in length. From 10 to 20 of these cells are grouped about a central axis much like the segments in an orange (Grenacher, 1879; see Fig. 7). Each appears to have a nerve fiber emerging from the base; this fiber runs proximally, converging with nerve fibers from neighboring retinula cells of the same and other ommatidia to form the optic nerve. The retinula cell is pigmented, except at the axial border. Here specialization into a laminated hyaline structure, the rhabdomere, is evident. The other neuroepithelial cell, of which there is usually only one in each ommatidium, is frequently termed the eccentric cell, from the observation that its cell body is situated on one side of the axis, near the proximal end of the sensory ommatidium (Watase, 1887; Demoll, 1917; see Fig. 8). It is distinguished in a number of other ways. There is a distal process that penetrates to the axis of the ommatidium and runs axially almost to the chitinous cone. It, too, has a nerve fiber that runs proximally with the nerve fibers of the surrounding retinula cells. Because this cell is not pigmented like the surrounding retinula cells and has a bipolar appearance, Watasé was tempted to refer to it as a ganglion cell. However, both he and Demoll regarded the eccentric cell as a sense cell and photoreceptor. Patten (1912) reported "a loose layer of ganglion cells lying just beneath the inner surface of the lateral eye." Grenacher *(loc. cit.)* could find no separate layer of ganglion cells.

The course of the nerve fibers after leaving the sensory ommatidia is not entirely clear. Behind the ommatidia there is a network of nerve fibers and connective tissue, that was termed by Watasé a "plexus." Indeed, Watasé felt that the bundles of nerve fibers from the ommatidia broke up in this plexus, made "peculiar" connections with other fibers, then recombined to form the optic nerve.

In our own preparations, the bundles of fibers emerging from the bases of the ommatidia can often be identified for a fair distance as they penetrate into the substance behind the eye. They can frequently be followed until they join together into larger bundles that can be seen ultimately to converge to form the optic nerve. Study of the optic nerve sections suggests that all of the sense cell fibers reach to and become part of the optic nerve (see also Waterman, *loc. cit.*). Still, the exact course of any individual nerve fiber is usually obscure in the net-

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FIGS. 6–8. Sections taken from lateral eye of *Limulus*. (From Wagner and Miller, in preparation; photomicrographs by W. H. Miller.)

FIG. 6. Horizontal section, showing seven entire ommatidia. Contrast between different corneal layers (C, C') and crystalline cones (CO) is exaggerated. Sections moderately bleached to show retinula (R). Only a portion of the plexus is shown (P). Lee Brown modification of Mallory's aniline blue stain.

FIG. 7. Cross section through sensory portion of an ommatidium, showing rosette configuration of retinula cells (RC). Light region in center is occupied by the rhabodomeres of the retinula cells, surrounding the central process of the eccentric cell. Portion of eccentric cell body shown on right (EC). Thionine stain; sections unbleached.

FIG. 8. Axial section through sensory portion of an ommatidium (corneal end at top). Shows eccentric cell body (EC), two retinula cells (RC) flanking the axial canal (light streak) in which is the central process of the eccentric cell. The rhabdomeres of the retinula cells appear as dark-staining bands bordering the axial canal. (Similar details may be seen in Fig. 6.) Lee Brown modification of Mallory's aniline blue stain; sections unbleached.

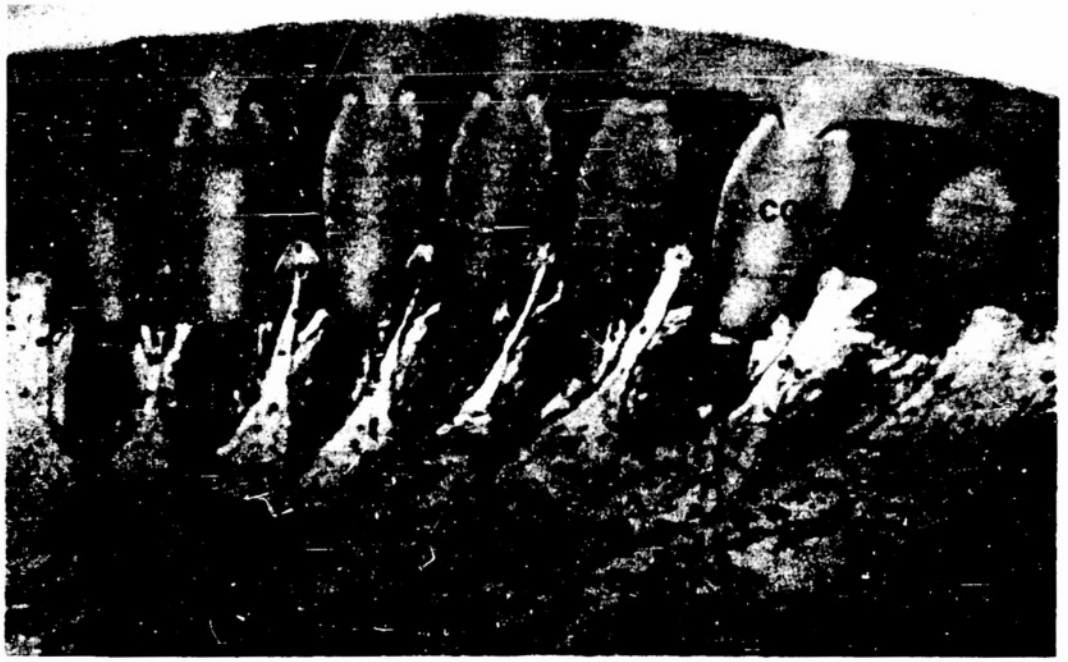

FIG. 6

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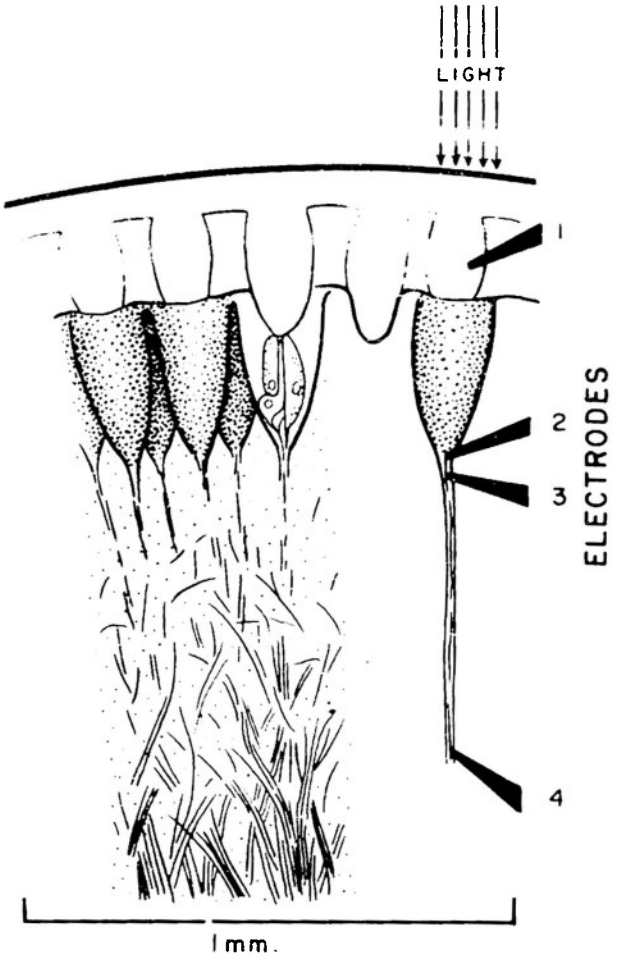

FIG. 9. Schematic drawing, representing a section of lateral eye of *Limulus* in a plane perpendicular to surface of cornea, as seen in fresh preparations. Transparent cornea at top, showing crystalline cones of the ommatidia; the heavily melanin-pigmented conical bodies of these form a layer on the inner surface of the cornea. On the left, a group of ommatidia is represented, with indications of bundles of nerve fibers traversing the plexus behind the ommatidia, collecting in larger bundles that become the optic nerve still farther back. One of these ommatidia has been represented as if the section had passed through it, revealing the sensory component, also as if sectioned. On the right an ommatidium with its nerve fiber bundle is represented as it appears after having been isolated by dissection and suspended, in air, on electrodes (moist cotton wicks, from chlorided silver tubes filled with seawater) represented by the solid black triangles.

work of fibers and cells that the bundles enter as soon as they leave the ommatidia. The histological detail of this region is difficult to determine. That some sort of functional connection exists between ommatidia we are confident, from physiological evidence that we shall present later in this paper. Although the anatomy of the *Limulus* eye is not as simple as early descriptions would lead one to believe, it is far less complex than the eyes of higher animals.

In our experiments on the isolated ommatidia of the *Limulus* eye we have begun by making a cut with a sharp razor blade through a freshly excised eye perpendicular to the corneal surface. From a little below the surface exposed by the cut a small nerve strand from one of the ommatidia can be snipped out with sharp, finely pointed scissors. If it has not been damaged by the dissection, such a bundle shows action potentials typical of the activity of a single nerve fiber, even though (as we have shown above) the nerve strand from each ommatidium contains ten or more fibers. We doubt that this simple response arises because all of the fibers discharge synchronously. From the rather uncertain evidence now at hand, we are inclined to believe that it is the activity of the axon from the eccentric cell that is recorded.

If the dissection has been successful up to this point, it is then possible to isolate the ommatidium from which this nerve strand arises by snipping away adjacent ommatidia, and finally stripping off all the rest of the tissue of the eye, leaving only this one ommatidium attached to the cornea. The situation is shown schematically in Figure 9. Fine cotton wicks leading to Ag-AgCl electrodes are then applied in the positions indicated. Leads 1 and 2 are taken to the input of a direct-coupled amplifier (which is always used for recording slow action potentials). Leads 3 and 4 are connected to a capacitance-coupled amplifier for the simultaneous recording of the action potential spikes in the nerve.

The slow, "retinal" action potential recorded from the body of an isolated ommatidium (Figs. 10, 13, 14) is a simple fluctuation in the potential difference between its ends, the distal (corneal) end becoming more negative with respect to the proximal end. We will term this the "ommatidial action potential." It begins suddenly after a latent period, rises steeply to a maximum and then subsides. At the same time that the ommatidial action potential starts to rise (or a little later) the discharge of impulses in the nerve fiber begins. The frequency of the discharge also rises to a maximum and then subsides.

The precise placement of the leads has an appreciable effect on the size of the ommatidial action potential that is recorded. To obtain the maximum response, the distal lead (1) should

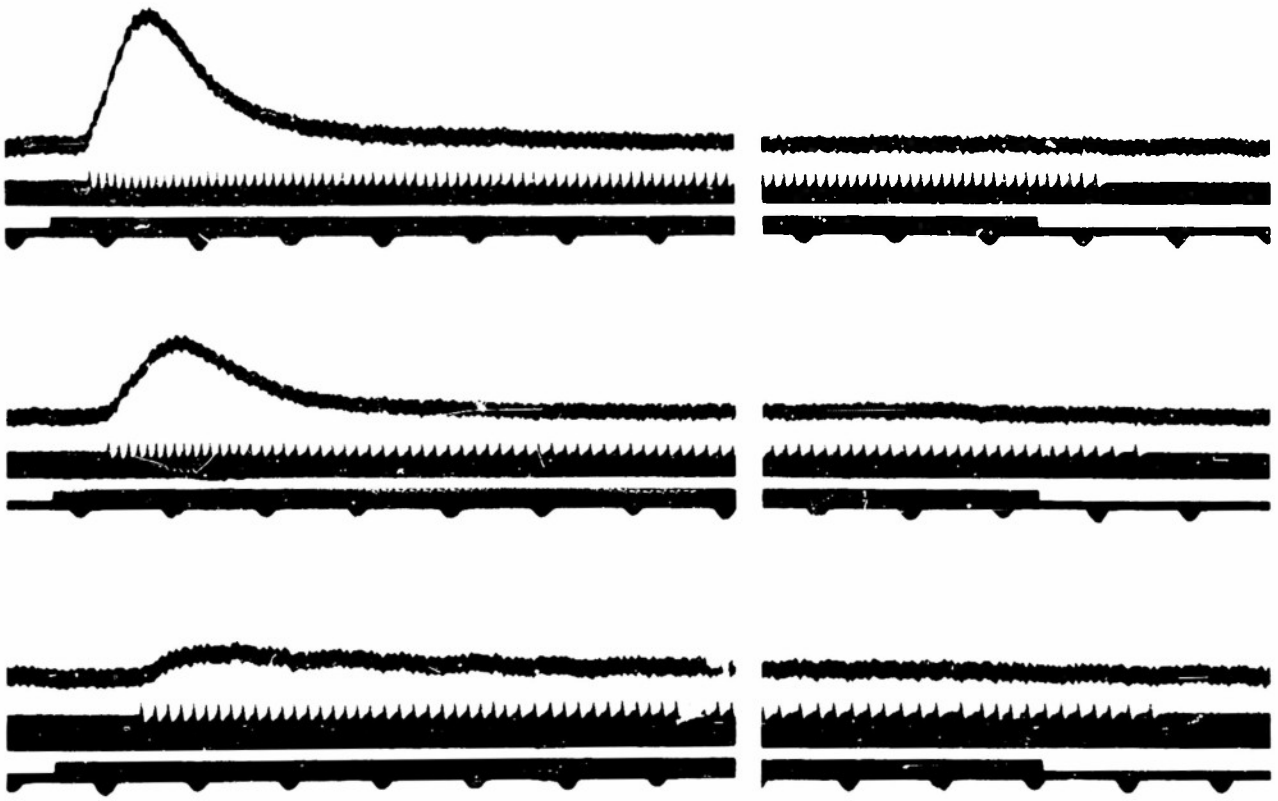

FIG. 10. Simultaneously recorded nerve and "retinal" action potentials of an isolated ommatidium from the eye of *Limulus*, in response to illumination at three intensities of relative value (top to bottom) 1.0, 0.1, 0.01. Upper trace in each record: action potential of the body of the ommatidium (leads 1-2, *cf.* Fig. 9), D. C. amplification. Lower trace (black edge) in each record: spike action potentials of the nerve strand from the ommatidium (amplifier time constant = 0.1 sec). For both traces, deflection upward indicates increasing negativity of distal leads (1, 3) with respect to proximal leads (2, 4). Peak deflection of upper trace in top record = 0.4 mv. In each record, signal marking period of illumination blackens lower half of white band above time marks. Time marked in $\frac{1}{5}$ sec.

be on the cornea, or close to it on the body of the ommatidium, and the proximal lead (2) should be near the proximal tip of the ommatidium. If part of the nerve strand is included between leads (1) and (2), spike potentials are recorded superimposed on the slow ommatidial potential (Fig. 11). Traces of these spikes are sometimes seen even when the proximal lead is on the ommatidium itself (see Figs. 13, 14). When some of the nerve is included with the ommatidium

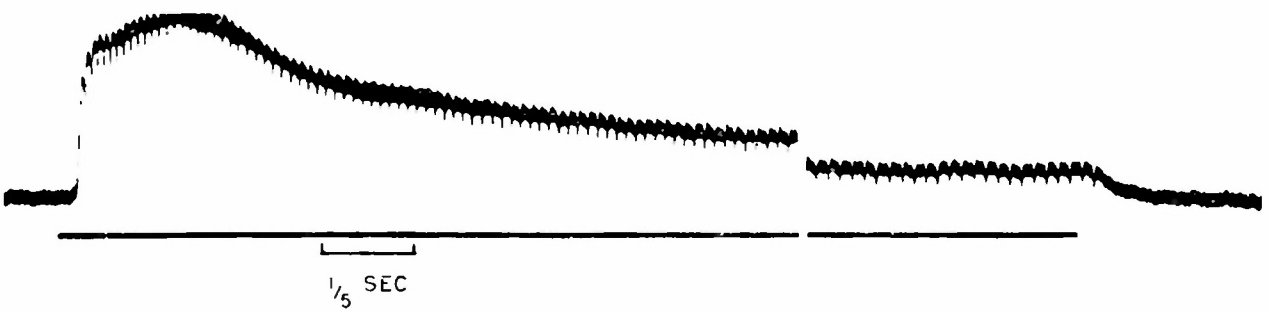

FIG. 11. Action potential of isolated ommatidium (*Limulus* eye) and its nerve strand (leads 1-4, Fig. 9) in response to prolonged steady illumination. Deflection upward indicates increasing negativity of cornea (lead 1) with respect to cut end of nerve strand (lead 4). D. C. amplification. Black line above time scale signals period of illumination (record interrupted for approx. 8 sec).

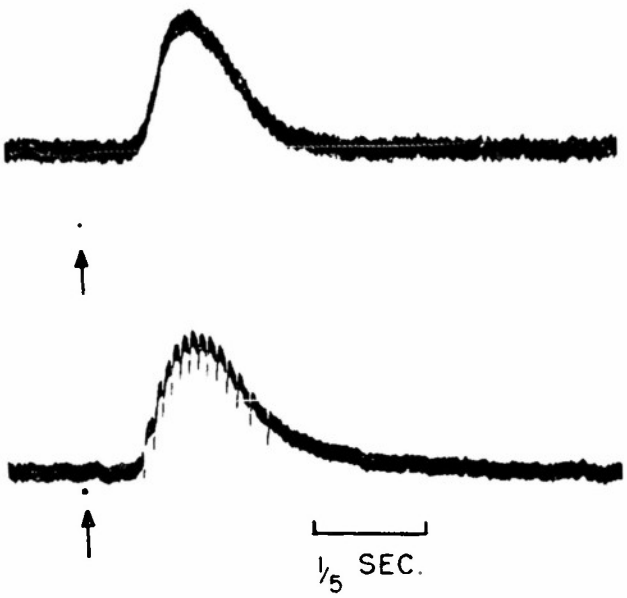

FIG. 12. Action potentials recorded from an isolated ommatidium and its nerve strand (leads 1-4; same preparation as in Fig. 11) in response to a flash of light 0.01 sec long (signals marked by arrows). Lower record: from intact ommatidium. Upper record: after piercing body of ommatidium with glass needle (somewhat higher amplification).

between leads 1 and 2, the action potential spikes are usually (but not always) in the downward direction on the records, indicating increased positivity of the distal lead with respect to the proximal lead; when only the nerve strand is between the electrodes (leads 3-4), the spike action potentials are in the usual direction, the distal lead becoming relatively more negative in the initial deflection.

Slight mechanical stretch of the ommatidium, or puncture with a fine glass needle, often results in a preparation that no longer discharges nerve impulses but still produces an ommatidial action potential in response to light (Fig. 12). More extensive mechanical disturbance will abolish all response; the preparation is very delicate.

The higher the intensity of the stimulating light, and, for short flashes, the longer its duration, the greater is the amplitude of the initial maximum of the slow action potential from an isolated ommatidium, and the higher the frequency of the discharge of impulses in its nerve strand in the initial outburst of activity (Figs. 10, 13). In response to test flashes of constant intensity, both the ommatidial action potential and the frequency of the discharge in the nerve fiber are decreased by light-adapting the receptor unit, and recover in a parallel manner during subsequent dark-adaptation (Fig. 14). Thus, the maximum amplitude of the ommatidial action potential and the maximum frequency reached in the corresponding discharge of impulses are closely correlated under various conditions of stimulation and adaptation. The slow action potentials recorded from the body of the ommatidium and the discharge of impulses in the nerve strand appear to be comparable manifestations of the initial phase of the activity of the sensory element.

However, comparison of these two signs of activity during the course of any single response reveals discrepancies that cannot be neglected. If the frequency of discharge is measured at various times during the course of a response, and compared with the corresponding values of the ommatidial action potential at these same times, it is seen (Fig. 15) that the two measures are not related in a simple manner. Indeed, a single value of potential is not uniquely associated with a single value of frequency. This is perhaps not too surprising, for the properties of the irritable mechanism may be expected to change during activity. However, in the later phases of the responses

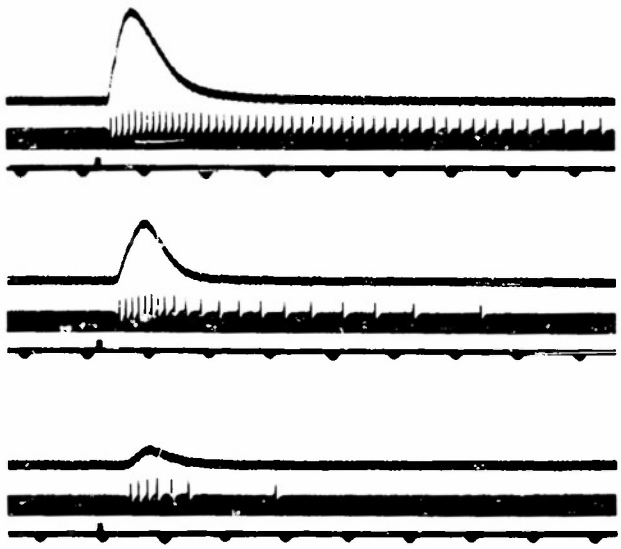

FIG. 13. Action potentials of an isolated ommatidium and its nerve strand recorded simultaneously, in response to short flashes of light (0.02 sec) at three intensities (relative value, top to bottom 1.0, 0.1, 0.01). Signal of light flash appears as black square near beginning of each record in lower half of white band just above time marks. Same preparation as in Fig. 10; see legend for details.

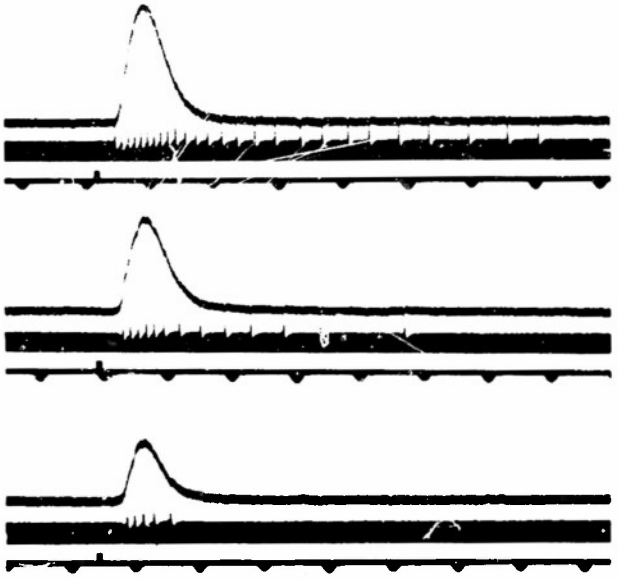

FIG. 14. Ommatidial and nerve fiber action potentials, recorded simultaneously from an isolated ommatidium *(Limulus)* in response to test flashes of fixed intensity at various times during dark adaptation (2 min., 4 min., 10 min., bottom to top) after a one minute exposure to a bright light. Same preparation as in Figs. 10 and 13; see legends for details.

to continued illumination the relation between the discharge of impulses and the level of potential appears to break down almost completely. After one or two seconds of illumination the potential difference between the ends of the ommatidium subsides almost to its original resting value, while the discharge of nerve impulses is maintained at a steady level as long as the light continues to shine. This is true even for high intensities that elicit a brisk discharge of impulses.

Limulus happens to be rather exceptional in showing only very slight elevations in retinal action potentials during steady illumination. The eyes of most other animals show distinct plateaus of potential, even when no ganglionic elements are present (Figs. 4 and 5). The retinal action potential of the cephalapod eye, in which the retina is a simple mosaic of receptor elements, is a particularly good example (Fröhlich, 1913; Therman, 1940). Perhaps in *Limulus*, steady potential gradients associated with steady nerve discharge develop in regions of the cellular elements not favorably oriented for recording by the arrangement of external leads that we have employed. Indeed, we have evidence of slow electrical processes that are associated with activity of the sensory element, but not recorded by leads confined to the body of the ommatidium. In records obtained with both the nerve strand and the body of the ommatidium included between the leads to the amplifier (Fig. 11) the level of the slow action potential can often be somewhat more satisfactorily correlated with frequency of impulse discharge than when the electrical response of the body of the ommatidium alone is considered (see Hartline, 1935). The nerve strand itself appears to contribute significantly to the potential gradients thus recorded. This contribution can be seen directly in some preparations, especially in those that have been slightly damaged so that the repetitive discharge of nerve impulses no longer takes place. When these slow potential changes in the nerve strand can be observed, their time course is very similar to the rise and fall of frequency of impulses discharged from undamaged preparations (Fig. 16), although no exact quantitative comparisons have yet been made.

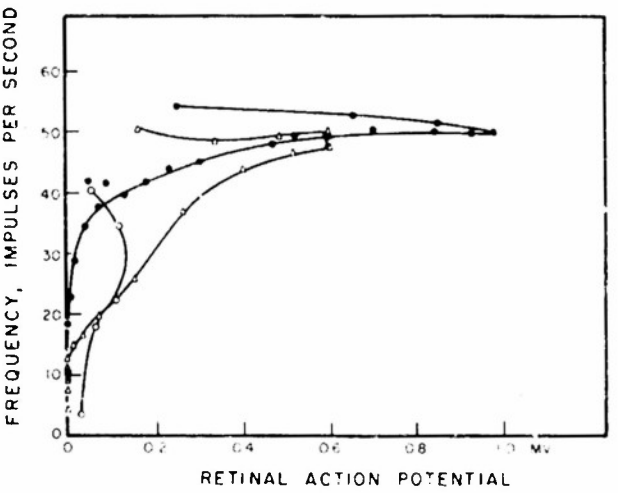

FIG. 15. Relation between the value of the slow action potential of an isolated ommatidium and the frequency of impulses in the nerve fiber attached to it, during the course of prolonged exposures to light. These curves were plotted from the records shown in Fig. 10 (● = top record, Δ = middle record; ○ = bottom record. See legend of Fig. 10 for details.) For each point the ordinate is the reciprocal of the time interval between successive impulses. The abscissa is the difference between the resting value of the retinal potential and its value at the time midway between these impulses. The upper left-hand point on each curve indicates the reciprocal of the interval between the first and second impulse in the discharge, and successive points indicate the frequencies determined by selected pairs of impulses during the course of the record.

The slow potential changes in the nerve strand, elicited by illumination of the receptor element, appear to be the result of electrotonic spread of electrical changes originating in the ommatidium. They resemble the electrotonic potentials recorded by Katz (1950) from the terminal nerve twigs of the muscle stretch receptor. Electrotonic spread of potential changes along nerve pathways from the receptor layer of the eye has been observed by other investigators (Bernhard, 1942; Parry, 1947); it may well contribute an important component of the total retinal action potential.

FIG. 16. Slow action potentials recorded from the nerve bundle attached to a single ommatidium that had been injured deliberately in such a way that nerve impulses were no longer discharged. The potential difference was obtained from a pair of wick electrodes, one of which was placed close to the point at which the nerve bundle emerged from the ommatidium; the other supported the cut end (electrode separation approx. 0.5 mm). The amplitude of the initial maximum of potential was 0.18 mv. (Deflection upward indicates electrode nearest ommatidium became more negative with respect to cut end.) The period of illumination is indicated by the black band above the time line (interval between time marks = ½ sec). After interruption of the record (approx. 5 sec), the light intensity was increased by 50%. (Signalled by upper black band.)

The isolated ommatidium of the *Limulus* eye affords an opportunity to study in detail the electrical responses of a structural element that appears to function as a single receptor unit. Still, our preparation does not consist of a single photoreceptor cell alone, and the electrical responses of the cell actually responsible for the discharge of nerve impulses may be partially masked by the activity of other cellular components of the retinula whose functions we do not understand (see Wulff, 1950). If, in addition, the geometrical arrangement of the cellular elements interferes with the faithful recording of the significant potential gradients, the interpretation of the action potentials recorded by external leads may be difficult, indeed. At best, in any preparation, the retinal action potential, as it is usually recorded, should be regarded only as an external manifestation of summated electrical events in the cellular components responsible for the generation of propagated impulses.

The recent development of micropipette electrodes small enough to penetrate single cells without killing them (Graham and Gerard, 1946; Ling and Gerard, 1949; Nastuk and Hodgkin, 1950; Brock, Coombs and Eccles, 1951) offers great promise of resolving some of the difficulties that we have encountered in identifying electrical processes leading to the generation of nerve impulses. Our own investigations along these lines are as yet preliminary. Examples of the electrical response recorded by a micropipette inserted into an isolated ommatidium are shown in Figures 17 and 18. Many probings by the pipette were necessary before the responses illustrated were obtained, even though the group of retinula cells always comprises a sizable fraction of the volume of the ommatidium. This would seem to support our belief that it is the eccentric cell that is responsible for the discharge of nerve impulses. In the experiment from which Figs. 17 and 18 were taken, the final successful probing resulted in a sudden change in the potential of the micropipette, the electrode becoming more negative with respect to an indifferent lead by at least 50 mv. At the same time, the nerve bundle from the ommatidium suddenly began to discharge impulses spontaneously, and, synchronously with each nerve impulse, spike-like positive deflections were recorded by the micropipette.

Upon illumination of the ommatidium (Fig. 17) the potential of the micropipette became less negative and remained at a nearly constant value, elevated above the resting level, as long as the light continued to shine. At the same time there was an increase in the frequency of the spikes recorded by means of the microelectrode and their concomitant impulses in the nerve. Upon cessation of illumination the frequency of discharge decreased and the potential of the microelectrode returned slowly to its resting value. The higher the intensity of the stimulating light, the greater was the elevation of potential of the microelectrode, and the greater was the increase in

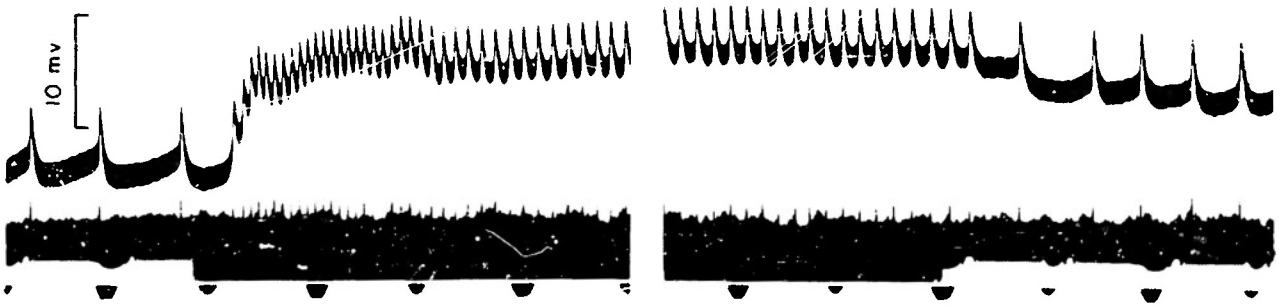

FIG. 17. Simultaneous records of the potentials arising within an ommatidium (upper trace) and from the nerve-bundle attached to the ommatidium (lower trace) in response to prolonged illumination. The black band under the lower trace indicates the duration of illumination. The activity of the ommatidium was recorded between a micropipette (tip diameter < 1 μ) inserted into it, and an indifferent electrode in the solution covering the eye. D. C. amplification was used; the resting potential having been cancelled by means of a potentiometer. Wick electrodes and a capacitance-coupled amplifier were used for recording the potentials from the nerve. Interval between time marks = ½ sec.

the frequency of the discharge of impulses. In response to a short flash (Fig. 18) there was a transitory increase in the potential of the microelectrode, and a simultaneous transitory increase in the frequency of the impulses. Both of these effects began after a latent period of .07 sec. It may be noted at this point that in Figure 17 the frequency of the impulses discharged by this element in response to light reached a high value initially, and then declined to a lower level that was maintained for the rest of the period of illumination, but that no corresponding maximum occurred in the initial development of the slow potential change. Our experience with this type of preparation is too limited to permit us to discuss the relation between the potential level and the frequency of nerve discharge, or to relate the potential changes recorded by means of the micropipette to the slow action potentials recorded with external electrodes on the ommatidium or its nerve bundle. However, we are encouraged to believe that the use of the micropipette enables us to observe directly a depolarization of the sensory element under the action of light, a depolarization that is intimately related to the initiation of nerve impulses and that is manifested externally as the retinal action potential.

Since electrical processes initiated by the action of light appear to be of importance in the generation of nervous activity, it is profitable to examine the effects of electrical current passed through the eye. An excised eye of *Limulus* was arranged so that current from a battery could be passed through it, while activity was recorded in a single optic nerve fiber (Hartline, Coulter and Wagner, 1952). Records from a typical experiment are shown in Figure 19. When the corneal surface of the eye was made cathodal, trains of impulses were discharged in the fiber during the passage of the current. When the cornea was made anodal, no impulses appeared in the nerve fiber during the time the current was flowing, but after it was stopped a burst of impulses was discharged. With the surface of the cornea cathodal, the discharges resembled those obtained in response to illumination of the eye, beginning at a high frequency and subsiding to a lower steady level. The stronger the exciting current, the shorter was the latency and the higher the frequency of the discharge, just as in the responses to stimulation by light. Only the latent period differed significantly: for responses of comparable frequency, the discharge always began in a much shorter time after the onset of the stimulating current than when light was the stimulus. When a current was passed through

FIG. 18. Simultaneous records of potentials arising within a single ommatidium (micropipette recording) and from the attached nerve-bundle in response to a short flash of light (0.02 sec). Other conditions as in Fig. 17.

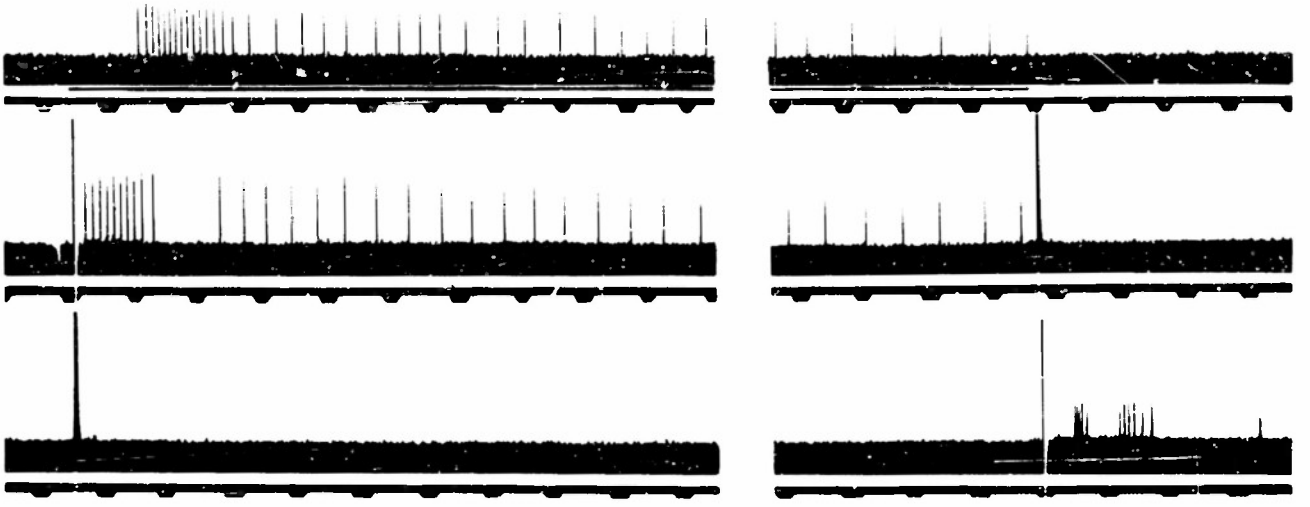

FIG. 19. Discharge of impulses in a single optic nerve fiber (*Limulus*, whole-eye preparation) in response to illumination and to electric current. Upper record: eye illuminated during the interval indicated by the black line in the white band above the timing line. Middle record: constant current (5 ma.) passed through the eye; cornea cathodal. Bottom record: constant current (5 ma.) passed through the eye; cornea anodal. Area of eye approximately 1 cm^2. The beginning and end of the constant currents were signalled in the middle and bottom records by the large artifacts near the start and finish of each record. A time-constant of 0.001 sec was used in the amplifier to avoid displacement of the base-line during the passage of current through the eye. (Time in $\frac{1}{5}$ sec). (From Hartline, Wagner and Coulter, in preparation.)

the eye in such a direction that the cornea was anodal, the burst of impulses that occurred after cessation of the current had a longer duration, a higher frequency and a shorter latency, the greater the intensity or duration (up to one or two seconds) of the stimulus.[2] Very similar responses to the passage of electric current have been observed in peripheral nerve by Fessard (1936), Arvanitaki (1938) and by Hodgkin (1948).

The effects of electric current passed through the eye of *Limulus* combine with the excitatory effects of light. In the experiments cited above, the frequency of discharge in a nerve fiber responding to steady illumination of the eye was increased when the cornea was made cathodal. Current passed in the opposite direction slowed the discharge, and if strong enough would stop it. During current flow in the inhibiting direction (cornea anodal) the threshold to a short flash of light was raised. Subliminal current, flowing in the exciting direction, (cornea cathodal) lowered the threshold to a flash of light, showing that the excitatory effects of subliminal current and subliminal light can summate to yield a response. So, evidently, light and electric current exert some of their effects at a common locus in the receptor or its nerve fiber.

Thus it appears that the visual receptors and their associated nerve fibers resemble other components of the nervous system, both in the action potentials they manifest when excited by their natural stimulus and in their reactions to applied electrical currents. Although many details remain to be clarified, it seems reasonable at the present time to predict that electrical aspects of the excitatory process in the photoreceptor will prove to play the same essential role as in the axons and cell bodies of neurones.

Even if we accept the hypothesis that the agent that generates activity in the afferent nerve fiber is an electrical process in the receptor, there still remains the question of how the initial photochemical reaction is linked to this electrical process. We may recall the well established fact that the retinal action potential, like the discharge of optic nerve impulses, does not begin immediately with the onset of illumination, but has an appreciable latency. Following a short flash of light it, too, may take place

[2] These responses following cessation of a current passed in a direction opposite to that causing excitation are of special interest, for they resemble "off" responses to sudden darkening of the eye that are often observed in visual systems, such as the distal retina of *Pecten*, (Hartline, 1938a) and certain of the ganglion cells of the Vertebrate retina (Hartline, 1938b; Granit, 1947). While elements responding to a decrease in illumination have never been observed in the lateral eye of *Limulus*, such responses have been found in the optic lobe of the central ganglion in this animal (Wilska and Hartline, 1941).

entirely in darkness (see Fig. 4, 12, 13, 14). This is universally true of retinal action potentials, whatever may be the animal form from which they are recorded. Simple eyes, in which there can be no question of ganglionic delays, show appreciable latencies in the appearance of the retinal potentials (see Fig. 4). In the isolated ommatidium of the *Limulus* eye, the retinal action potential recorded by external leads does not begin to rise until just slightly before the first nerve impulse of the response is discharged (see especially Figs. 11 and 12). The same is true of the electrical responses recorded by a micropipette in the ommatidium (Figs. 17, 18). Analysis of the latent period of the retinal potential of the isolated ommatidium shows that it, like the latent period of the optic nerve discharge, has a "critical duration." Thus there must be an appreciable time lag between photolysis and the beginning of the electrical response. The short latency of the nerve response to an applied electrical current, as contrasted with that of a comparable response to light, suggests that much of the delay in the generation of nerve impulses must be attributed to the elaboration of products of photolysis, and to their action in initiating the basic electrical process that in turn is assumed to generate nerve activity. Recently Wald and Brown (1952) have made the specific suggestion that the liberation of sulf-hydryl groups in the bleaching of rhodopsin may generate electrical potential gradients in the receptor, but it is still too early to judge whether this will account for the observed facts of receptor excitation. The links between the special photochemical mechanism of the visual receptor and the more general neural excitation processes remain obscure.

A comprehensive discussion of the properties of a sensory system would not be complete without reference to the complex neural interactions that take place between component cells. In a paper restricted to the consideration of the peripheral origin of nervous activity such a topic might properly be excluded, were it not for the fact that in some sensory systems, the very origin of nervous activity in the most peripheral receptor unit is affected by such interactions. This is true in the eye of *Limulus*, as the next section shows, and has also been reported for the sensory structure of the cochlea of the Vertebrate ear (Galambos, 1944). The principle involved may well be of general importance.

In the compound lateral eye of *Limulus*, activity in a single optic nerve fiber can be elicited by illumination of one, and only one, ommatidium. Nevertheless, if a given ommatidium is illuminated steadily, giving rise to a steady train of impulses in its nerve fiber, illumination of other regions of the eye not too far distant from it produces a pronounced slowing of the discharge (Fig. 20; Hartline, 1949). The brighter the light on these regions, and the larger the area illuminated, the grenter is this inhibiting effect upon the discharge. The effect becomes weaker for regions farther removed from the specific ommatidium, but often extends for a radius of several millimeters, over a third or more of the total area of the eye. If the activity is recorded from nerve fibers from two ommatidia not too widely separated, it can be shown that the inhibitory effect is reciprocal, each one affecting the other. Thus the activity of each ommatidium inhibits, and in turn is inhibited by, activity of many other ommatidia in surrounding regions of the eye.

We do not yet understand how this inhibitory action is exerted, except that it appears to be dependent on the integrity of the nervous pathways in the network of fibers back of the ommatidia. In a few experiments, we have been able to show that if the bundle of nerve fibers from an ommatidium is snipped out, as in the first step of isolating an ommatidium (described above), the inhibitory effects are thereby abolished. Moreover, a microelectrode inserted into the ommatidium itself, thus recording the discharge of impulses at their point of origin, shows the same slowing of the rate of discharge when

FIG. 20. Inhibition of the activity of a receptor element by illumination of a nearby retinal area. A single optic nerve fiber (*Limulus* whole-eye preparation) was caused to discharge impulses by shining a small spot of light on the cornea, focussed upon the ommatidium of the fiber. The illumination had commenced 5 sec. before the start of the record and continued throughout its duration. During the period indicated by the blackening of the white line above the time record a region of the cornea several millimeters distant from the excited ommatidium was illuminated. (Time in ¼ sec).

inhibiting areas of the eye are illuminated, provided the connections back of the ommatidia are intact.

Although we cannot explain the mechanism of this inhibitory influence in the *Limulus* eye, it is easy to understand its role in visual function. Since the inhibition of any receptor element is greater the higher the intensity of light shining on the surrounding regions, it is evident that brightly illuminated areas of the eye inhibit the activity of dimly lighted regions more than the latter inhibit the activity of the former. Thus contrast is enhanced. By a relatively simple type of interaction among elements of the eye an important visual effect is achieved.

For this paper the significance of the interaction observed in the eye of *Limulus* is that the degree of nervous activity initiated by any single photoreceptor unit is determined not only by the conditions of stimulation and adaptation of that unit, but also by the degree of activity of adjacent receptors. Each individual sensory element does not function as an isolated detecting device for luminous energy, totally independent of the action of all its fellows. The process of integration of nervous activity may extend peripherally to the very elements in which that activity is generated.

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DISCUSSION

MONNIER: Among the remarkable results obtained by Dr. Hartline one appeared to me particularly striking. It is the parallelism between the subliminal photoexcitation of a receptor and the subliminal electrical excitation of a nerve. This observation indicates that both modes of excitation bear upon the same processes. It is therefore possible that photoexcitation may disclose oscillatory phenomena just as those shown in certain cases, by electrical stimulation. This situation seems to appear in human vision. The apparent brilliancy of a source of light presents a marked overshoot above its normal value, at the beginning of the illumination. This overshoot is more and more apparent as the true brilliancy increases, and is finally followed by several oscillations. This oscillatory behavior has been accurately accounted for by the late Dr. Lassalle according to the kinetics of the photo-chemical cycle of reactions which appear to be actually involved.

HARTLINE: The excitability of the *Limulus* photoreceptor unit shows a marked "overshoot" at the beginning of a period of prolonged subliminal illumination, but after this initial maximum the excitability subsides to a steady level, greater than its value in darkness, without any oscillations that we have been able to detect. However, the responses to supra-liminal excitation of the dark-adapted, isolated ommatidium usually show a minimum in the frequency of the discharge of nerve impulses following the initial maximum. This is more pronounced the higher the intensity of the stimulating light. Sometimes a small second maximum is present before the discharge settles down to a steady frequency. Thus, as Dr. Monnier suggests, these photoreceptors do show oscillatory phenomena, but the oscillations are heavily damped.

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